Stability of Steviol Glycosides in Full-Fat and Skimmed Set Yoghurt

Jooken, E.*, Amery R., Meesschaert, B.
KHBO, Chemistry Department, Faculty of Industrial Science and Technology
Zeedijk 101, B-8400 Oostende, Belgium

Introduction

In our modern day society, the metabolic syndrome is a major concern to our health care. The main causes are lack of physical exercise and an unbalanced diet with too much added sugar. Non-caloric sweeteners can be important to decrease the caloric intake. Stevia is a non-caloric sweetener of natural origin which may fulfill an important role in countering the metabolic syndrome. Stevia is the extract of the leaves of *Stevia Rebaudiana*, a perennial shrub indigenous to South America. The sweet tasting compounds are known as steviol glycosides (SVGly). The most important ones are Rebaudioside A (RebA) and Stevioside (Ste)[1]. The final approval for stevia as an additive to food is expected by the end of 2011 [2].

The authors demonstrated earlier [3] that soil bacteria from Paraguay are able to hydrolyze SVGly to the diterpene aglycon steviol, i.e. they have β-glycosidase activity. Yoghurt contains live microorganisms which have obviously β-galactosidase enzymes, because they hydrolyze lactose. The question that needs answering is now whether these microorganisms also have β-glycosidase activity. Or, in other words, are SVGly stable in yoghurt?

Materials and methods

Full-fat and skimmed set yoghurt were processed in the pilot plant of ILVO (Institute for Agriculture and Fisheries Research, Melle, Belgium). A sugar reduction of 30% compared to the reference of 7.3 % sugar was obtained by the addition of a mixture of SVGly (chiefly Ste, RebA and RebC plus some minor SVGly) or nearly pure RebA. The yoghurt was packaged immediately after inoculation with the starter culture and incubated at 43 °C until pH 4.65 was reached. The samples were stored at 6 °C for 35 days and analyzed intermittently.

Prior to analysis, fat was removed by centrifugation and proteins precipitated by addition of acetonitrile and centrifuged again. Next, the steviol glycosides were concentrated on a solid phase extraction cartridge. The final analysis was done by HPLC (Thermo-Fischer) on a C18 stationary phase and an aqueous phosphoric acid – acetonitrile gradient as the mobile phase and a detection wavelength of 200 nm[4]. Recovery (based on RebA) was 102 %.

Results

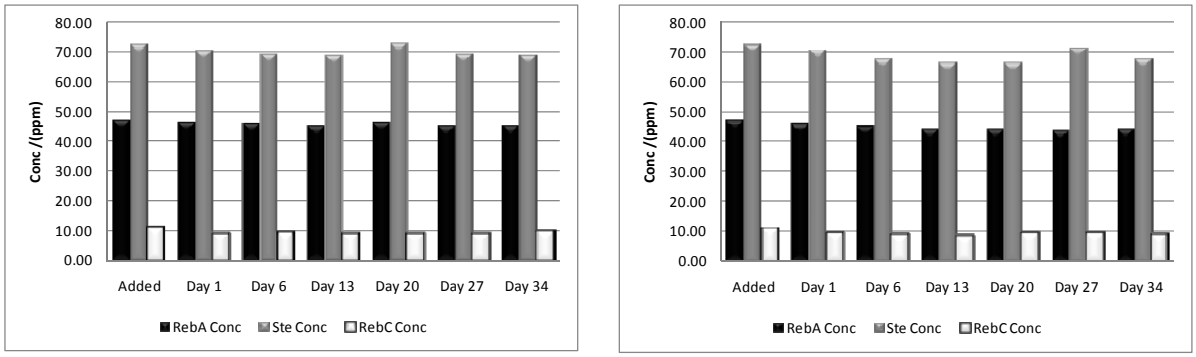


Figure 1: Analysis of SVGly mixture in full-fat set yoghurt (left) and skimmed set yoghurt (right)

Discussion

The results for the addition of the SVGly mixture show good stability, in both full-fat and skimmed yoghurt (see Figure 1). The results for the addition of nearly pure RebA were analogous, but are not shown here. Although the bacteria in the yoghurt have β-galactosidase activity, apparently they lack the necessary β-glucosidase enzymes that are necessary to hydrolyze the SVGly. We can also conclude from these results that the pH is not low enough to cause chemical hydrolysis of the glycosidic bonds.

The final conclusion is that SVGly are suitable for sweetening yoghurt products, and thus can contribute to a decrease in caloric intake.

References

1. J. M. C. Geuns. *Phytochemistry* **64**, 913-921 (2003).
2. EFSA. *EFSA Journal* **8**, 1537 (2010).
3. A. Smedts, R. Amery, N. Moons, E. Jooken, B. Meesschaert. In *Proceedings of the 2nd Stevia Symposium 2008: Steviol glycosides: technical and pharmacological aspects* (J. M. C. Geuns, ed.), pp. 5-27. Euprint Ed., Leuven (2008).
4. R. Amery, E. Jooken, B. Duquenne, J. Geuns, B. Meesschaert. In *Proceedings of the 4th Stevia Symposium 2010 organized by EUSTAS: Stevia: science, no fiction* (J. Geuns, ed.), pp. 69-81. Euprint, Leuven (2010).

Stability of Steviol Glycosides in Full-Fat and Skimmed Set Yoghurt

R. Amery([1]), B. Duquenne([2]), E. Jooken*([1,4]), J. Geuns([3]), B. Meesschaert([1,4])

([1]) KHBO, Fac Ind Sci & Techn, Chem Dept, Zeedijk 101, B-8400 Oostende
([2]) Institute for Agricultural and Fisheries Research (ILVO), Technology and FoodScience Unit, Brusselsesteenweg 370, B-9090 Melle, Belgium
([3]) Department of Biology, Laboratory of Functional Biology KU Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium
([4]) Centre for Surface Chemistry and Catalysis And Leuven Food Science and Nutrition Research Centre (LForCe) Department of Microbial and Molecular Systems KU Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

1. Introduction

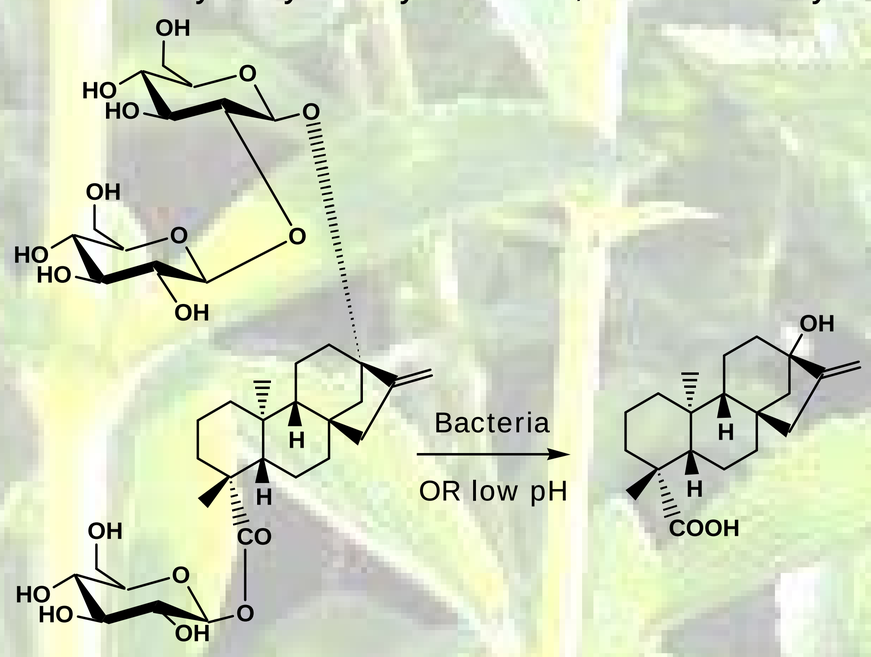
Metabolic Syndrome is a major problem in modern times. Apart from physical excercise, sugar intake in food needs to be reduced.

Obesitas
High Blood Pressure
High Triglycerides
Low HDL-cholesterol
Insulin Resistance

Steviol Glycosides (SVGly's) are non-caloric, natural sweeteners extracted from the leaves of Stevia Rebaudiana.

SVGly's have been used for many years in South America, Japan, China, Australia. Approval in EU is expected by the end of this year.

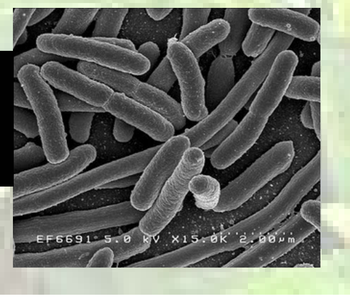
SVGly's can be hydrolysed by bacteria, or chemically at low pH.



stevioside (one of the main SvGly's)
200 x sweeter than sucrose

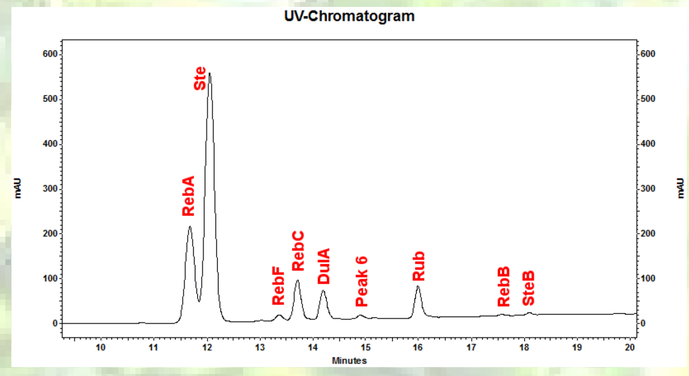
Steviol
not sweet

Question: are SVGly stable in set yoghurt (pH = 4 ; live bacteria)?



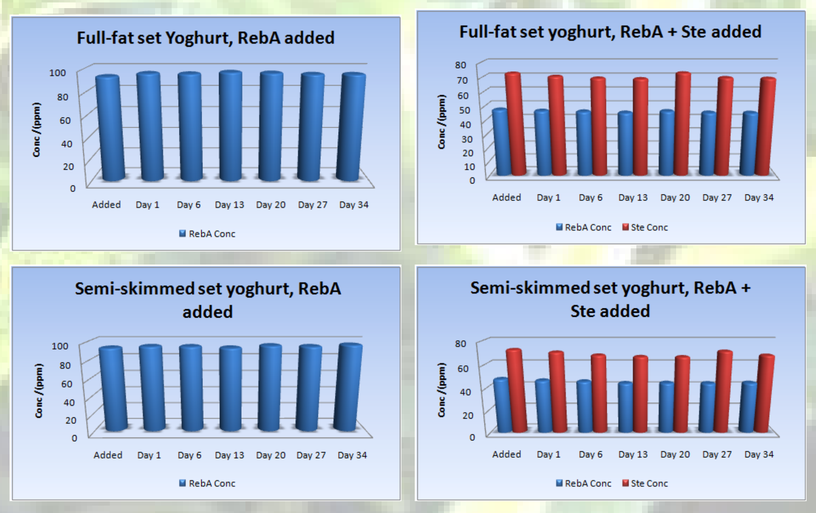
2. Experimental

Samples of full-fat and semi-skimmed set yoghurt were processed at ILVO's pilot plant
Sample prep included centrifugation (to remove fat and proteins) and solid phase extraction (to concentrate the SVGly's). Analysis was done by HPLC on C18 column.



Samples were stored for 35 days at 6 °C, and analyzed periodically. Either Rebaudioside A (RebA) or a mixture of RebA and Stevioside (Ste) was added to the samples (Amery, 2011).

3. Results and discussion



The SvGly's show no indication of break-down in any of the samples and under the storage conditions used.

4. Acknowledgements

The authors thank IWT-Vlaanderen for a research grant, the companies that contributed and Flanders FOOD for the coordination of the project.

5. References

Amery, R., Struyf, T., Duquenne, B., Jooken, E., Geuns, J., Meesschaert, B., 2011. Determination of Steviol Glycosides in Various Food Categories. In: Geuns, J. (Ed.), Proceedings of the 5th Stevia symposium. Euprint, Leuven, pp. 153-165.

* Corresponding author: etienne.jooken@khbo.be